invention be limited by the extent of the deficiency. The present invention contemplates various structures, including altered structures (primary, secondary, etc.), as well as native structures, that may be inhibited by synthesis inhibitors.

Where the polymerase structure is altered, it is not intended that the invention be limited by the means by which the structure is altered. In one embodiment, the alteration of the native DNA sequence comprises a change in a single nucleotide. In another embodiment, the alteration of the native DNA sequence comprises a deletion of one or more nucleotides. In yet another embodiment, the alteration of the native DNA sequence comprises an insertion of one or more nucleotides. It is contemplated that the change in DNA sequence may manifest itself as change in amino acid sequence.

The present invention contemplates structure-specific nucleases from a variety of sources, including mesophilic, psychrophilic, thermophilic, and hyperthermophilic organisms. The preferred structure-specific nucleases are thermostable. Thermostable structure-specific nucleases are contemplated as particularly useful in that they operate at temperatures where nucleic acid hybridization is extremely specific, allowing for allele-specific detection (including single-base mismatches). In one embodiment, the thermostable structure-specific are thermostable 5' nucleases which are selected from the group consisting of altered polymerases derived from the native polymerases of *Thermus* species, including, but not limited to *Thermus aquaticus*, *Thermus flavus,* and *Thermus thermophilus*. However, the invention is not limited to the use of thermostable 5' nucleases. Thermostable structure-specific nucleases from the FEN-1, RAD2 and XPG class of nucleases are also preferred.

The present invention provides a composition comprising a cleavage structure, said cleavage structure comprising: a) a target nucleic acid, said target nucleic acid having a first region, a second region, a third region and a fourth region, wherein said first region is located adjacent to and downstream from said second region, said second region is located adjacent to and downstream from said third region and said third region is located adjacent to and downstream from said fourth region; b) a first oligonucleotide complementary to said fourth region of said target nucleic acid; c) a

second oligonucleotide having a 5' portion and a 3' portion wherein said 5' portion of said second oligonucleotide contains a sequence complementary to said second region of said target nucleic acid and wherein said 3' portion of said second oligonucleotide contains a sequence complementary to said third region of said target nucleic acid; and d) a third oligonucleotide having a 5' portion and a 3' portion wherein said 5' portion of said third oligonucleotide contains a sequence complementary to said first region of said target nucleic acid and wherein said 3' portion of said third oligonucleotide contains a sequence complementary to said second region of said target nucleic acid.

The present invention is not limited by the length of the four regions of the target nucleic acid. In one embodiment, the first region of the target nucleic acid has a length of 11 to 50 nucleotides. In another embodiment, the second region of the target nucleic acid has a length of one to three nucleotides. In another embodiment, the third region of the target nucleic acid has a length of six to nine nucleotides. In yet another embodiment, the fourth region of the target nucleic acid has a length of 6 to 50 nucleotides.

The invention is not limited by the nature or composition of the of the first, second, third and fourth oligonucleotides; these oligonucleotides may comprise DNA, RNA, PNA and combinations thereof as well as comprise modified nucleotides, universal bases, adducts, etc. Further, one or more of the first, second, third and the fourth oligonucleotides may contain a dideoxynucleotide at the 3' terminus.

In a preferred embodiment, the target nucleic acid is not completely complementary to at least one of the first, the second, the third and the fourth oligonucleotides. In a particularly preferred embodiment, the target nucleic acid is not completely complementary to the second oligonucleotide.

As noted above, the present invention contemplates the use of structure-specific nucleases in a detection method. In one embodiment, the present invention provides a method of of detecting the presence of a target nucleic acid molecule by detecting non-target cleavage products comprising: a) providing: i) a cleavage means, ii) a source of target nucleic acid, the target nucleic acid having a first region, a second region, a third region and a fourth region, wherein the first region is located adjacent to and

downstream from the second region, the second region is located adjacent to and downstream from the third region and the third region is located adjacent to and downstream from the fourth region; iii) a first oligonucleotide complementary to the fourth region of the target nucleic acid; iv) a second oligonucleotide having a 5' portion and a 3' portion wherein the 5' portion of the second oligonucleotide contains a sequence complementary to the second region of said target nucleic acid and wherein the 3' portion of the second oligonucleotide contains a sequence complementary to the third region of the target nucleic acid; iv) a third oligonucleotide having a 5' and a 3' portion wherein the 5' portion of the third oligonucleotide contains a sequence complementary to the first region of the target nucleic acid and wherein the 3' portion of the third oligonucleotide contains a sequence complementary to the second region of the target nucleic acid; b) mixing the cleavage means, the target nucleic acid, the first oligonucleotide, the second oligonucleotide and the third oligonucleotide to create a reaction mixture under reaction conditions such that the first oligonucleotide is annealed to the fourth region of the target nucleic acid and wherein at least the 3' portion of the second oligonucleotide is annealed to the target nucleic acid and wherein at least the 5' portion of the third oligonucleotide is annealed to the target nucleic acid so as to create a cleavage structure and wherein cleavage of the cleavage structure occurs to generate non-target cleavage products, each non-target cleavage product having a 3'-hydroxyl group; and c) detecting the non-target cleavage products.

The invention is not limited by the nature of the target nucleic acid. In one embodiment, the target nucleic acid comprises single-stranded DNA. In another embodiment, the target nucleic acid comprises double-stranded DNA and prior to step c), the reaction mixture is treated such that the double-stranded DNA is rendered substantially single-stranded. In another embodiment, the target nucleic acid comprises RNA and the first and second oligonucleotides comprise DNA.

The invention is not limited by the nature of the cleavage means. In one embodiment, the cleavage means is a structure-specific nuclease; particularly preferred structure-specific nucleases are thermostable structure-specific nucleases. In a preferrred embodiment, the thermostable structure-specific nuclease is encoded by a